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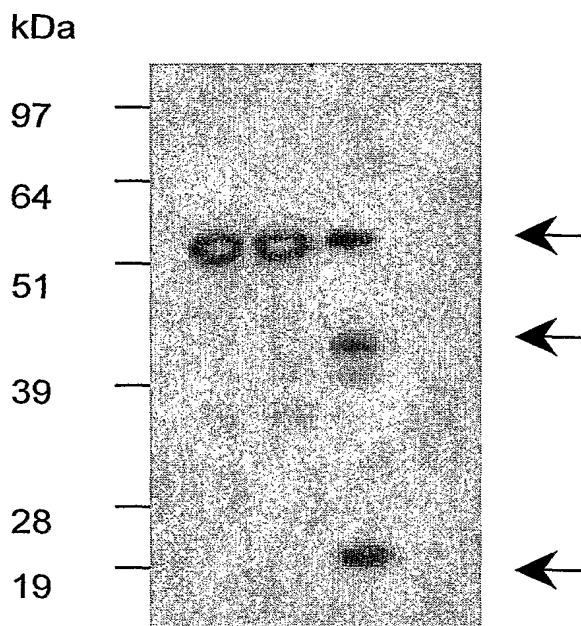
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(54) Title: LACTOFERRIN CLEAVAGE OF NEISSERIAL PROTEINS



(57) Abstract: Meningococcal antigens are cleaved by human lactoferrin. The invention is based on the identification of the cleavage products of this reaction, and provides a method for cleaving a neisserial polypeptide, comprising the step of mixing the polypeptide with a lactoferrin enzyme. The invention also provides polypeptides obtainable by this process (i.e. the cleavage products of the lactoferrin digestion). Proteins of particular interest are meningococcal proteins 287 and App.

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LACTOFERRIN CLEAVAGE OF NEISSERIAL PROTEINS

All documents cited herein are incorporated by reference in their entirety.

TECHNICAL FIELD

This invention is in the field of proteolysis. In particular, it relates to the proteolytic cleavage of antigens of *Neisseria meningitidis* (meningococcus).

BACKGROUND ART

N.meningitidis is a non-motile, Gram-negative human pathogen that colonises the pharynx and causes meningitis (and, occasionally, septicaemia in the absence of meningitis). It causes both endemic and epidemic disease. Following the introduction of the conjugate vaccine against *Haemophilus influenzae*, *N.meningitidis* is the major cause of bacterial meningitis in the USA.

Based on the organism's capsular polysaccharide, various serogroups of *N.meningitidis* have been identified, including A, B, C, H, I, K, L, 29E, W135, X, Y & Z. Serogroup A (MenA) is the pathogen most often implicated in epidemic disease in sub-Saharan Africa. Serogroups B and C (MenB & MenC) are responsible for the vast majority of cases in the United States and in most developed countries. Serogroups W135 and Y are responsible for the rest of the cases in the USA and developed countries. Although the capsular polysaccharide is an effective protective immunogen for some serogroups, this approach is unsuitable for immunising against serogroup B.

For serogroup B, a vaccine has proved elusive. Vaccines based on outer-membrane vesicles have been tested, but protection is typically restricted to the strain that was used to make the vaccine (homologous protection). Research and development on serogroup B vaccines continues [1], with a focus on recombinant and subunit vaccines.

Genome sequences for meningococcal serogroups A [2] and B [3,4] have been reported, and the serogroup B sequence has been studied to identify vaccine antigens [e.g. refs. 5 to 10]. Candidate antigens have been manipulated to improve heterologous expression [refs. 11 to 13].

These antigens have been characterised at the level of primary sequence, and many have also been studied for post-translational processing e.g. NarE [14], NadA [15], App [16], GNA1870 [17], etc. However, it is not known how the proteins behave after administration to patients.

DISCLOSURE OF THE INVENTION

Surprisingly, the inventors have found that meningococcal antigens are cleaved by human lactoferrin.

The invention is based on the identification of the cleavage products of this reaction.

Thus the invention provides a method for cleaving a neisserial polypeptide, comprising the step of mixing the polypeptide with a lactoferrin enzyme. The invention also provides polypeptides obtainable by this process (i.e. the cleavage products of the lactoferrin digestion).

Neisseria are known to bind to lactoferrin and to display lactoferrin receptors [MenB genes NMB1540 & NMB1541; refs. 18-24], but lactoferrin-mediated cleavage of neisserial proteins has not been reported. Cleavage of *H.influenzae* proteins by lactoferrin has been reported [25,26], but this bacterium is not closely related to *Neisseria* and there is no reason to expect that a similar activity would be seen with *Neisseria*.

The neisserial polypeptide

Genome sequences for serogroups A [2] and B [3,4] of meningococcus have been published, and a genome sequence for *N.gonorrhoeae* (gonococcus) strain FA 1090 is available on-line [27,28].

The neisserial polypeptide cleaved by lactoferrin according to the invention can be any of the polypeptide sequences encoded and/or expressed by a *Neisseria*, including *N.meningitidis*, *N.gonorrhoeae*, *N.lactamica*, *N.polysaccharea*, *N.subflava*, *N.sicca* and *N.cinerea*. The polypeptide is preferably from *N.meningitidis* or *N.gonorrhoeae*.

Preferred *N.meningitidis* polypeptides are those known as 'NadA', '936', '953', '287', 'App', 'TonBR' and 'NarE'. Particularly preferred are '287' and 'App'.

App protein

'App' protein (Adhesion and penetration protein) from serogroup B meningococcus is disclosed in reference 5 (SEQ IDs 647-654) as 'ORF1', as NMB1985 in reference 3 (see also GenBank accession number GI:7227246), and as 'BASB006' in ref. 29. The corresponding protein in serogroup A [2] has GenBank accession number 7379205. App is a serine protease.

When used according to the present invention, App protein may take various forms. Preferred forms of App are truncation or deletion variants, such as those disclosed in reference 30, or the substitution mutants also disclosed in reference 30 (substitutions at Ser-267, Asp-158 and His-115).

Preferred App sequences have 50% or more identity (e.g. 60%, 70%, 80%, 90%, 95%, 99% or more) to SEQ ID NO: 1. This includes App variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.). Other preferred App sequences comprise at least *n* consecutive amino acids from SEQ ID NO: 1, wherein *n* is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments comprise an epitope from App. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from either or both of the C-terminus and/or the N-terminus of SEQ ID NO: 1.

App has Arg-rich regions (see residues 933-941 (SEQ ID NO: 22) and 1150-1156 (SEQ ID NO: 23) of SEQ ID NO: 1) that are highly conserved between different strains, and lactoferrin-catalysed cleavage occurs within one or both of these regions.

287 protein

'287' protein from serogroup B is disclosed in reference 7 (SEQ IDs 3103 & 3104), as 'NMB2132' in reference 3, and as 'GNA2132' in reference 10 (see also GenBank accession number GI:7227388). The corresponding protein in serogroup A [2] has GenBank accession number 7379057.

- 5 When used according to the present invention, 287 protein may take various forms. Preferred forms of 287 are truncation or deletion variants, such as those disclosed in references 11 to 13. In particular, the N-terminus of 287 may be deleted up to and including its poly-glycine sequence (*i.e.* deletion of residues 1 to 24 for strain MC58), which is sometimes distinguished herein by the use of a 'ΔG' prefix. This deletion can enhance expression.
- 10 Preferred 287 sequences have 50% or more identity (*e.g.* 60%, 70%, 80%, 90%, 95%, 99% or more) to SEQ ID NO: 2. This includes 287 variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*). Allelic forms of 287 can be seen in Figures 5 and 15 of reference 9, and in example 13 and figure 21 of reference 7 (SEQ IDs 3179 to 3184). Other preferred 287 sequences comprise at least *n* consecutive amino acids from SEQ ID NO: 2, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16,
- 15 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments comprise an epitope from 287. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from either or both of the C-terminus and/or the N-terminus of SEQ ID NO: 2.

- Protein 287 has an Arg-rich region (see residues 237-245 (SEQ ID NO: 21) of SEQ ID NO: 2) which
- 20 is highly conserved across strains. The site of lactoferrin-mediated cleavage is within this region.

NadA protein

'NadA' (Neisserial adhesin A) from serogroup B of *N.meningitidis* is disclosed as protein '961' in reference 7 (SEQ IDs 2943 & 2944) and as 'NMB1994' in reference 3 (see also GenBank accession numbers: 11352904 & 7227256). A detailed description of the protein can be found in reference 15.

- 25 There is no corresponding protein in serogroup A [2,15].

When used according to the present invention, NadA may take various forms. Preferred forms of NadA are truncation or deletion variants, such as those disclosed in references 11 to 13. In particular, NadA without its C-terminal membrane anchor is preferred (*e.g.* deletion of residues 351-405 for strain 2996), which is sometimes distinguished herein by the use of a 'C' superscript *e.g.* NadA^(C).

- 30 Expression of NadA without its membrane anchor domain in *E.coli* results in secretion of the protein into the culture supernatant with concomitant removal of its 23mer leader peptide (*e.g.* to leave a 327mer for strain 2996). Polypeptides without their leader peptides are sometimes distinguished herein by the use of a 'NL' superscript *e.g.* NadA^(NL) or NadA^{(C)(NL)}.

- Preferred NadA sequences have 50% or more identity (*e.g.* 60%, 70%, 80%, 90%, 95%, 99% or
- 35 more) to SEQ ID NO: 3. This includes NadA variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*). Allelic forms of NadA are shown in Figure 9 of reference 30. Other

preferred NadA sequences comprise at least n consecutive amino acids from SEQ ID NO: 3, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments comprise an epitope from NadA. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from either or both of the C-terminus and/or the N-terminus of SEQ ID NO: 3 (e.g. NadA^(C), NadA^(NL), NadA^{(C)(NL)}). Where N-terminus residues are deleted, it is preferred that the deletion should not remove the ability of NadA to adhere to human epithelial cells..

936 protein

'936' protein from serogroup B is disclosed in reference 7 (SEQ IDs 2883 & 2884) and as 'NMB2091' in reference 3 (see also GenBank accession number GI:7227353). The corresponding gene in serogroup A [2] has GenBank accession number 7379093.

When used according to the present invention, 936 protein may take various forms. Preferred forms of 936 are truncation or deletion variants, such as those disclosed in references 11 to 13. In particular, the N-terminus leader peptide of 936 may be deleted (i.e. deletion of residues 1 to 23 for strain MC58 [SEQ ID 4]) to give 936^(NL).

Preferred 936 sequences have 50% or more identity (e.g. 60%, 70%, 80%, 90%, 95%, 99% or more) to SEQ ID NO: 4. This includes variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants etc). Other preferred 936 sequences comprise at least n consecutive amino acids from SEQ ID NO: 4, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments comprise an epitope from 936. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from either or both of the C-terminus and/or the N-terminus of SEQ ID NO: 4.

953 protein

'953' protein from serogroup B is disclosed in reference 7 (SEQ IDs 2917 & 2918) and as 'NMB1030' in reference 3 (see also GenBank accession number GI:7226269). The corresponding protein in serogroup A [2] has GenBank accession number 7380108.

When used according to the present invention, 953 protein may take various forms. Preferred forms of 953 are truncation or deletion variants, such as those disclosed in references 11 to 13. In particular, the N-terminus leader peptide of 953 may be deleted (i.e. deletion of residues 1 to 19 for strain MC58 [SEQ ID 5]) to give 953^(NL).

Preferred 953 sequences have 50% or more identity (e.g. 60%, 70%, 80%, 90%, 95%, 99% or more) to SEQ ID NO: 5. This includes 953 variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.). Allelic forms of 953 can be seen in Figure 19 of reference 9. Other preferred 953 sequences comprise at least n consecutive amino acids from SEQ ID NO: 5, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments comprise an epitope from 953. Other preferred fragments lack one or more

amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from either or both of the C-terminus and/or the N-terminus of SEQ ID NO: 5.

'TonBR'

'TonBR' protein from serogroup B meningococcus is disclosed as 'NMB1882' in reference 3 (see also GenBank accession number GI:7227141). The corresponding protein in serogroup A [2] has GenBank accession number 7379311 [2]. TonBR is the TonB-dependent receptor (or ferric siderophore receptor).

Preferred TonBR sequences have 50% or more identity (*e.g.* 60%, 70%, 80%, 90%, 95%, 99% or more) to SEQ ID NO: 11. This includes TonBR variants (*e.g.* allelic variants, homologs, orthologs, paralog, mutants, *etc.*). Other preferred TonBR sequences comprise at least *n* consecutive amino acids from SEQ ID NO: 11, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments comprise an epitope from TonBR. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from either or both of the C-terminus and/or the N-terminus of SEQ ID NO: 11.

NarE protein

'NarE' protein from serogroup B meningococcus is disclosed as NMB1343 in reference 3 (see also GenBank accession number GI:7226587). There is no corresponding protein in serogroup A [2]. NarE is an ADP ribosyltransferase [16].

Preferred NarE sequences have 50% or more identity (*e.g.* 60%, 70%, 80%, 90%, 95%, 99% or more) to SEQ ID NO: 6. This includes NarE variants (*e.g.* allelic variants, homologs, orthologs, paralog, mutants, *etc.*). Other preferred NarE sequences comprise at least *n* consecutive amino acids from SEQ ID NO: 6, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments comprise an epitope from NarE. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from either or both of the C-terminus and/or the N-terminus of SEQ ID NO: 6. Polymorphic forms of NarE are shown in Figure 2 of ref. 13.

Other meningococcal proteins

Other proteins which may be cleaved by lactoferrin according to the invention include those comprising one of the following amino acid sequences: SEQ ID NO:650 from ref. 5; SEQ ID NO:878 from ref. 5; SEQ ID NO:884 from ref. 5; SEQ ID NO:4 from ref. 6; SEQ ID NO:598 from ref. 7; SEQ ID NO:818 from ref. 7; SEQ ID NO:864 from ref. 7; SEQ ID NO:866 from ref. 7; SEQ ID NO:1196 from ref. 7; SEQ ID NO:1272 from ref. 7; SEQ ID NO:1274 from ref. 7; SEQ ID NO:1640 from ref. 7; SEQ ID NO:1788 from ref. 7; SEQ ID NO:2288 from ref. 7; SEQ ID NO:2466 from ref. 7; SEQ ID NO:2554 from ref. 7; SEQ ID NO:2576 from ref. 7; SEQ ID NO:2606 from ref. 7; SEQ ID NO:2608 from ref. 7; SEQ ID NO:2616 from ref. 7; SEQ ID NO:2668 from ref. 7; SEQ ID NO:2780 from ref. 7; SEQ ID NO:2932 from ref. 7; SEQ ID NO:2958 from ref. 7; SEQ ID

NO:2970 from ref. 7; SEQ ID NO:2988 from ref. 7, or a polypeptide comprising an amino acid sequence which: (a) has 50% or more identity (e.g. 60%, 70%, 80%, 90%, 95%, 99% or more) to said sequences; and/or (b) comprises a fragment of at least *n* consecutive amino acids from said sequences, wherein *n* is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more).

The lactoferrin

Lactoferrin is a Fe^{3+} -binding glycoprotein, first recognized in milk and then in other human epithelial secretions and barrier fluids. Many different functions have been attributed to LF, including protection from iron-induced lipid peroxidation, immunomodulation and cell growth regulation, DNA binding, and transcriptional activation. Its physiological role is still unclear, but it has been suggested to be responsible for primary defense against microbial and viral infection. Different subfractions of purified human milk LF possess five different enzyme activities: DNase, RNase, ATPase, phosphatase, and malto-oligosaccharide hydrolysis [31]. Lactoferrin is the predominant source of these activities in human milk. Some of its catalytically active sub-fractions are cytotoxic and induce apoptosis. The enzyme can be purified from milk or expressed recombinantly. Purified lactoferrin can be obtained from various commercial suppliers.

The lactoferrin used in the method of the invention is preferably a human lactoferrin. As well as cleaving neisserial polypeptides, this enzyme has been found to cleave *H.influenzae* surface proteins at Arg-rich regions [32].

Cleavage products

The invention also provides the cleavage products of the lactoferrin-mediated proteolysis of the invention. These fragments can be prepared by lactoferrin proteolysis, of course, or can be prepared by other means in the "already-cleaved" form. Their preparation may or may not involve proteolytic cleavage, depending on the method chosen. They may be prepared by purification from cell culture, recombinant expression, chemical synthesis (at least in part), etc. The cleavage products can take various forms (e.g. native, fusions, non-glycosylated, lipidated, etc.). They are preferably prepared in substantially pure form (i.e. substantially free from other *N.meningitidis* or host cell proteins).

A preferred cleavage product comprises an amino acid sequence of formula -A1-B1-C1-, where: A1 is an optional arginine residue; B1 is a sequence with 50% or more (e.g. 60%, 70%, 80%, 90% or 100%) sequence identity to SEQ ID NO: 7; and C1 is any amino acid sequence. Sequence C1 preferably has 50% or more (e.g. 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100%) sequence identity to SEQ ID NO: 8. The -A1-B1-C1- sequence may be at the N-terminus of a polypeptide, at the C-terminus, or may have sequences both to its C- and N-termini.

Another cleavage product comprises an amino acid sequence of formula -A2-B2-, where: B2 is an optional arginine residue; and A2 is a sequence with 50% or more (e.g. 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100%) sequence identity to SEQ ID NO: 9 and/or SEQ ID NO: 10. The

-A2-B2- sequence may be at the N-terminus of a polypeptide, at the C-terminus, or may have sequences both to its C- and N-termini.

Where -B1- is SEQ ID NO: 7 and -C1- is SEQ ID NO: 8, -A1-B1-C1- is a proteolytic fragment of meningococcal protein 287. In this case, the optional -A1- moiety is absent in 70% of cleavages.

- 5 Where -A2- is SEQ ID NO: 9, -A2-B2- is a proteolytic fragment of meningococcal protein 287. In this case, the optional -B2- moiety is absent in 30% of cleavages. Thus the sequence -A2-B2-A1-B1-C1- is protein '287' (SEQ ID NO: 2), where -B2-A1- is a single Arg residue. Where -A2- is SEQ ID NO: 10, -A2-B2-A1-B1-C1- is 'ΔG287'.

- 10 Another preferred cleavage product comprises an amino acid sequence of formula -A3-B3-C3-, where: A3 is an optional arginine residue; B3 is a sequence with 50% or more (*e.g.* 60%, 70%, 80%, 90% or 100%) sequence identity to SEQ ID NO: 12; and C3 is any amino acid sequence. Sequence C3 preferably has 50% or more (*e.g.* 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100%) sequence identity to SEQ ID NO: 13. The -A3-B3-C3- sequence may be at the N-terminus of a polypeptide, at the C-terminus, or may have sequences both to its C- and N-termini.

- 15 Another preferred cleavage product comprises an amino acid sequence of formula -A4-B4-C4-, where: A4 is an optional arginine residue; B4 is a sequence with 50% or more (*e.g.* 60%, 70%, 80%, 90% or 100%) sequence identity to SEQ ID NO: 14; and C4 is any amino acid sequence. Sequence C4 preferably has 50% or more (*e.g.* 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100%) sequence identity to SEQ ID NO: 15. The -A4-B4-C4- sequence may be at the N-terminus of a polypeptide, at the C-terminus, or may have sequences both to its C- and N-termini.
- 20

- Another cleavage product comprises an amino acid sequence of formula -A5-B5-, where: B5 is an optional arginine residue; and A5 is a sequence with 50% or more (*e.g.* 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100%) sequence identity to SEQ ID NO: 16. The -A5-B5- sequence may be at the N-terminus of a polypeptide, at the C-terminus, or may have sequences both to its C- and N-termini.
- 25

- Another cleavage product comprises an amino acid sequence of formula -A6-B6-, where: B6 is an optional arginine residue; and A6 is a sequence with 50% or more (*e.g.* 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100%) sequence identity to SEQ ID NO: 17. The -A6-B6- sequence may be at the N-terminus of a polypeptide, at the C-terminus, or may have sequences both to its C- and N-termini.
- 30

These polypeptides are cleavage products of App. SEQ ID NO: 1 can be made up of -A5-B5-A3-B3-C3-, where -B5-A3- is a single Arg residue. Alternatively, it can be made up of -A6-B6-A4-B4-C4-, where -B6-A4- is a single Arg residue.

- Each of -A2-, -C1-, -C3-, -C4-, -A5- and -A6- preferably consists of at least 10 amino acids (*e.g.* at least 12, 14, 16, 18, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 400, 500 or more).
- 35

Preferably the polypeptide comprising sequence -A1-B1-C1- does not include the amino acid sequence SEQ ID NO: 18 and/or SEQ ID NO: 21. Preferably the polypeptide comprising sequence -A2-B2- does not include the amino acid sequence SEQ ID NO: 7. Preferably the polypeptide comprising sequence -A3-B3-C3- does not include the amino acid sequence SEQ ID NO: 19 and/or
5 SEQ ID NO: 22. Preferably the polypeptide comprising sequence -A4-B4-C4- does not include the amino acid sequence SEQ ID NO: 20 and/or SEQ ID NO: 23. Preferably the polypeptide comprising sequence -A5-B5- does not include the amino acid sequence SEQ ID NO: 12. Preferably the polypeptide comprising sequence -A6-B6- does not include amino acid sequence SEQ ID NO: 14.

10 The invention also provides nucleic acid encoding cleavage products. Furthermore, the invention provides nucleic acid which can hybridise to this nucleic acid, preferably under "high stringency" conditions (*e.g.* 65°C in a 0.1xSSC, 0.5% SDS solution).

Nucleic acid according to the invention can be prepared in many ways (*e.g.* by chemical synthesis (at least in part), from genomic or cDNA libraries, from the organism itself, *etc.*) and can take various forms (*e.g.* single stranded, double stranded, vectors, probes, *etc.*). They are preferably prepared in
15 substantially pure form (*i.e.* substantially free from other *N.meningitidis* or host cell nucleic acids).

The term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones (*e.g.* phosphorothioates, *etc.*), and also peptide nucleic acids (PNA) *etc.* The invention includes nucleic acid comprising sequences complementary to those described above (*e.g.* for antisense or probing purposes).

20 The invention also provides a process for producing a polypeptide of the invention, comprising the step of culturing a host cell transformed with nucleic acid of the invention under conditions which induce polypeptide expression.

The invention provides a process for producing a polypeptide of the invention, comprising the step of synthesising at least part of the polypeptide by chemical means.

25 The invention provides a process for producing nucleic acid of the invention, comprising the step of amplifying nucleic acid using a primer-based amplification method (*e.g.* PCR).

The invention provides a process for producing nucleic acid of the invention, comprising the step of synthesising at least part of the nucleic acid by chemical means.

Strains

30 Meningococcal proteins cleaved by lactoferrin can be taken from any strain, and the use of a particular strain for exemplification herein should not be seen as limiting.

Preferred proteins of the invention comprise an amino acid sequence found in *N.meningitidis* serogroup B. Within serogroup B, preferred strains are 2996, MC58, 95N477, and 394/98. Strain 394/98 is sometimes referred to herein as 'NZ', as it is a New Zealand strain.

Protein 287 is preferably from strain 2996 or, more preferably, from strain 394/98.

Proteins 936, 953 and NadA are preferably from strain 2996.

Strains may be indicated as a subscript *e.g.* NadA_{MC58} is protein NadA from strain MC58. Unless otherwise stated, proteins mentioned herein (*e.g.* with no subscript) are from *N.meningitidis* strain 2996, which can be taken as a 'reference' strain. It will be appreciated, however, that the invention is not in general limited by strain. As mentioned above, general references to a protein (*e.g.* '287', '919' *etc.*) may be taken to include that protein from any strain. This will typically have sequence identity to 2996 of 90% or more (*e.g.* 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more).

Where a composition includes a particular protein antigen (*e.g.* 287 or NadA), the composition can include that antigen in more than one variant form *e.g.* the same protein, but from more than one strain. These proteins may be included as tandem or separate proteins.

Heterologous host

Whilst expression of the proteins of the invention may take place in *Neisseria*, the present invention preferably utilises a heterologous host. The heterologous host may be prokaryotic (*e.g.* a bacterium) or eukaryotic. It is preferably *E.coli*, but other suitable hosts include *Bacillus subtilis*, *Vibrio cholerae*, *Salmonella typhi*, *Salmonella typhimurium*, *Neisseria lactamica*, *Neisseria cinerea*, *Mycobacteria* (*e.g.* *M.tuberculosis*), yeast, *etc.*

Immunogenic compositions and medicaments

The invention provides an immunogenic composition comprising a polypeptide of the invention. Such compositions are preferably vaccine compositions. Vaccines according to the invention may either be prophylactic (*i.e.* to prevent infection) or therapeutic (*i.e.* to treat infection), but will typically be prophylactic.

The pH of the composition is preferably between 6 and 8, preferably about 7. Stable pH may be maintained by the use of a buffer. Where a composition comprises an aluminium hydroxide salt, it is preferred to use a histidine buffer [33]. The composition may be sterile and/or pyrogen-free. Compositions of the invention may be isotonic with respect to humans.

Compositions may be presented in vials, or they may be presented in ready-filled syringes. The syringes may be supplied with or without needles. A syringe will include a single dose of the composition, whereas a vial may include a single dose or multiple doses. Injectable compositions will usually be liquid solutions or suspensions. Alternatively, they may be presented in solid form (*e.g.* freeze-dried) for solution or suspension in liquid vehicles prior to injection.

Compositions of the invention may be packaged in unit dose form or in multiple dose form. For multiple dose forms, vials are preferred to pre-filled syringes. Effective dosage volumes can be

routinely established, but a typical human dose of the composition for injection has a volume of 0.5ml.

Where a composition of the invention is to be prepared extemporaneously prior to use (*e.g.* where a component is presented in lyophilised form) and is presented as a kit, the kit may comprise two vials, or it may comprise one ready-filled syringe and one vial, with the contents of the syringe being used to reactivate the contents of the vial prior to injection.

The invention also provides a composition of the invention for use as a medicament. The medicament is preferably able to raise an immune response in a mammal (*i.e.* it is an immunogenic composition) and is more preferably a vaccine.

The invention also provides the use of a composition of the invention in the manufacture of a medicament for raising an immune response in a mammal. It also provides the use of a polypeptide of the invention in the manufacture of a medicament for raising an immune response in a mammal. The medicament is preferably a vaccine.

The invention also provides a method for raising an immune response in a mammal comprising the step of administering an effective amount of a composition of the invention. The immune response is preferably protective and preferably involves antibodies. The method may raise a booster response.

The mammal is preferably a human. Where the vaccine is for prophylactic use, the human is preferably a child (*e.g.* a toddler or infant); where the vaccine is for therapeutic use, the human is preferably an adult. A vaccine intended for children may also be administered to adults *e.g.* to assess safety, dosage, immunogenicity, *etc.*

These uses and methods are preferably for the prevention and/or treatment of a disease caused by a *Neisseria* (*e.g.* meningitis, septicaemia, bacteremia, gonorrhoea *etc.*). The prevention and/or treatment of bacterial or meningococcal meningitis is preferred.

One way of checking efficacy of therapeutic treatment involves monitoring *Neisserial* infection after administration of the composition of the invention. One way of checking efficacy of prophylactic treatment involves monitoring immune responses against antigens after administration of the composition. Immunogenicity of compositions of the invention can be determined by administering them to test subjects (*e.g.* children 12-16 months age, or animal models [34]) and then determining standard parameters including serum bactericidal antibodies (SBA) and ELISA titres (GMT) of total and high-avidity IgG. These immune responses will generally be determined around 4 weeks after administration of the composition, and compared to values determined before administration of the composition. A SBA increase of at least 4-fold or 8-fold is preferred. Where more than one dose of the composition is administered, more than one post-administration determination may be made.

Preferred compositions of the invention can confer an antibody titre in a patient that is superior to the criterion for seroprotection for each antigenic component for an acceptable percentage of human

subjects. Antigens with an associated antibody titre above which a host is considered to be seroconverted against the antigen are well known, and such titres are published by organisations such as WHO. Preferably more than 80% of a statistically significant sample of subjects is seroconverted, more preferably more than 90%, still more preferably more than 93% and most preferably 96-100%.

- 5 Compositions of the invention will generally be administered directly to a patient. Direct delivery may be accomplished by parenteral injection (*e.g.* subcutaneously, intraperitoneally, intravenously, intramuscularly, or to the interstitial space of a tissue), or by rectal, oral, vaginal, topical, transdermal, intranasal, ocular, aural, pulmonary or other mucosal administration. Intramuscular administration to the thigh or the upper arm is preferred. Injection may be via a needle (*e.g.* a
10 hypodermic needle), but needle-free injection may alternatively be used. A typical intramuscular dose is 0.5 ml.

The invention may be used to elicit systemic and/or mucosal immunity.

- Dosage treatment can be a single dose schedule or a multiple dose schedule. Multiple doses may be used in a primary immunisation schedule and/or in a booster immunisation schedule. A primary dose
15 schedule may be followed by a booster dose schedule. Suitable timing between priming doses (*e.g.* between 4-16 weeks), and between priming and boosting, can be routinely determined.

- Neisserial infections affect various areas of the body and so the compositions of the invention may be prepared in various forms. For example, the compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles
20 prior to injection can also be prepared (*e.g.* a lyophilised composition). The composition may be prepared for topical administration *e.g.* as an ointment, cream or powder. The composition may be prepared for oral administration *e.g.* as a tablet or capsule, or as a syrup (optionally flavoured). The composition may be prepared for pulmonary administration *e.g.* as an inhaler, using a fine powder or a spray. The composition may be prepared as a suppository or pessary. The composition may be
25 prepared for nasal, aural or ocular administration *e.g.* as spray, drops, gel or powder [*e.g.* 35 & 36].

- Immunogenic compositions used as vaccines comprise an immunologically effective amount of antigen(s), as well as any other components, as needed. By 'immunologically effective amount', it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and
30 physical condition of the individual to be treated, age, the taxonomic group of individual to be treated (*e.g.* non-human primate, primate, *etc.*), the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials, and a typical quantity of
35 each antigen per dose is between 1µg and 20µg *e.g.* about 1µg, about 2.5µg, about 4µg, about 5µg, or about 10µg.

The composition of the invention will typically, in addition to the components mentioned above, comprise one or more 'pharmaceutically acceptable carriers', which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolised macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, sucrose [37], trehalose [38], lactose, and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of ordinary skill in the art. The vaccines may also contain diluents, such as water, saline, glycerol, *etc.* Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present. Sterile pyrogen-free, phosphate-buffered physiologic saline is a typical carrier. A thorough discussion of pharmaceutically acceptable excipients is available in reference 39.

Compositions of the invention may include an antimicrobial, particularly when packaged in multiple dose format.

Compositions of the invention may comprise detergent *e.g.* a Tween (polysorbate), such as Tween 80. Detergents are generally present at low levels *e.g.* <0.01%.

Compositions of the invention may include sodium salts (*e.g.* sodium chloride) to give tonicity. A concentration of 10±2mg/ml NaCl is typical.

Compositions of the invention will generally include a buffer. A phosphate buffer is typical.

Compositions of the invention may comprise a sugar alcohol (*e.g.* mannitol) or a disaccharide (*e.g.* sucrose or trehalose) *e.g.* at around 15-30mg/ml (*e.g.* 25 mg/ml), particularly if they are to be lyophilised or if they include material which has been reconstituted from lyophilised material. The pH of a composition for lyophilisation may be adjusted to around 6.1 prior to lyophilisation.

Vaccines of the invention may be administered in conjunction with other immunoregulatory agents. In particular, compositions will usually include an adjuvant. Adjuvants which may be used in compositions of the invention include, but are not limited to:

A. Mineral-containing compositions

Mineral containing compositions suitable for use as adjuvants in the invention include mineral salts, such as aluminium salts and calcium salts. The invention includes mineral salts such as hydroxides (*e.g.* oxyhydroxides), phosphates (*e.g.* hydroxyphosphates, orthophosphates), sulphates, *etc.* [*e.g.* see chapters 8 & 9 of ref. 40], or mixtures of different mineral compounds, with the compounds taking any suitable form (*e.g.* gel, crystalline, amorphous, *etc.*), and with adsorption being preferred. The mineral containing compositions may also be formulated as a particle of metal salt [41].

Aluminium phosphates are particularly preferred, particularly in compositions which include a *H.influenzae* saccharide antigen, and a typical adjuvant is amorphous aluminium hydroxyphosphate with PO₄/Al molar ratio between 0.84 and 0.92, included at 0.6mg Al³⁺/ml. Adsorption with a low

dose of aluminium phosphate may be used *e.g.* between 50 and 100µg Al³⁺ per conjugate per dose. Where there is more than one conjugate in a composition, not all conjugates need to be adsorbed.

B. Oil Emulsions

Oil emulsion compositions suitable for use as adjuvants in the invention include squalene-water emulsions, such as MF59 [Chapter 10 of ref. 40; see also ref. 42] (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer). Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) may also be used.

C. Saponin formulations [chapter 22 of ref. 40]

Saponin formulations may also be used as adjuvants in the invention. Saponins are a heterologous group of sterol glycosides and triterpenoid glycosides that are found in the bark, leaves, stems, roots and even flowers of a wide range of plant species. Saponin from the bark of the *Quillaia saponaria* Molina tree have been widely studied as adjuvants. Saponin can also be commercially obtained from *Smilax ornata* (sarsapilla), *Gypsophilla paniculata* (brides veil), and *Saponaria officianalis* (soap root). Saponin adjuvant formulations include purified formulations, such as QS21, as well as lipid formulations, such as ISCOMs. QS21 is marketed as Stimulon™.

Saponin compositions have been purified using HPLC and RP-HPLC. Specific purified fractions using these techniques have been identified, including QS7, QS17, QS18, QS21, QH-A, QH-B and QH-C. Preferably, the saponin is QS21. A method of production of QS21 is disclosed in ref. 43. Saponin formulations may also comprise a sterol, such as cholesterol [44].

Combinations of saponins and cholesterol can be used to form unique particles called immunostimulating complexes (ISCOMs) [chapter 23 of ref. 40]. ISCOMs typically also include a phospholipid such as phosphatidylethanolamine or phosphatidylcholine. Any known saponin can be used in ISCOMs. Preferably, the ISCOM includes one or more of Quila, QHA & QHC. ISCOMs are further described in refs. 44-46. Optionally, the ISCOMS may be devoid of additional detergent [47].

A review of the development of saponin based adjuvants can be found in refs. 48 & 49.

D. Virosomes and virus-like particles

Virosomes and virus-like particles (VLPs) can also be used as adjuvants in the invention. These structures generally contain one or more proteins from a virus optionally combined or formulated with a phospholipid. They are generally non-pathogenic, non-replicating and generally do not contain any of the native viral genome. The viral proteins may be recombinantly produced or isolated from whole viruses. These viral proteins suitable for use in virosomes or VLPs include proteins derived from influenza virus (such as HA or NA), Hepatitis B virus (such as core or capsid proteins), Hepatitis E virus, measles virus, Sindbis virus, Rotavirus, Foot-and-Mouth Disease virus, Retrovirus, Norwalk virus, human Papilloma virus, HIV, RNA-phages, Qβ-phage (such as coat proteins), GA-phage, fr-phage, AP205 phage, and Ty (such as retrotransposon Ty protein p1). VLPs are discussed further in refs. 50-55. Virosomes are discussed further in, for example, ref. 56

E. Bacterial or microbial derivatives

Adjuvants suitable for use in the invention include bacterial or microbial derivatives such as non-toxic derivatives of enterobacterial lipopolysaccharide (LPS), Lipid A derivatives, immunostimulatory oligonucleotides and ADP-ribosylating toxins and detoxified derivatives thereof.

- 5 Non-toxic derivatives of LPS include monophosphoryl lipid A (MPL) and 3-O-deacylated MPL (3dMPL). 3dMPL is a mixture of 3 de-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains. A preferred "small particle" form of 3 De-O-acylated monophosphoryl lipid A is disclosed in ref. 57. Such "small particles" of 3dMPL are small enough to be sterile filtered through a 0.22µm membrane [57]. Other non-toxic LPS derivatives include monophosphoryl lipid A mimics, such as
10 aminoalkyl glucosaminide phosphate derivatives *e.g.* RC-529 [58,59].

Lipid A derivatives include derivatives of lipid A from *Escherichia coli* such as OM-174. OM-174 is described for example in refs. 60 & 61.

- Immunostimulatory oligonucleotides suitable for use as adjuvants in the invention include nucleotide sequences containing a CpG motif (a dinucleotide sequence containing an unmethylated cytosine
15 linked by a phosphate bond to a guanosine). Double-stranded RNAs and oligonucleotides containing palindromic or poly(dG) sequences have also been shown to be immunostimulatory.

- The CpG's can include nucleotide modifications/analogues such as phosphorothioate modifications and can be double-stranded or single-stranded. References 62, 63 and 64 disclose possible analog substitutions *e.g.* replacement of guanosine with 2'-deoxy-7-deazaguanosine. The adjuvant effect of
20 CpG oligonucleotides is further discussed in refs. 65-70.

The CpG sequence may be directed to TLR9, such as the motif GTCGTT or TTCGTT [71]. The CpG sequence may be specific for inducing a Th1 immune response, such as a CpG-A ODN, or it may be more specific for inducing a B cell response, such a CpG-B ODN. CpG-A and CpG-B ODNs are discussed in refs. 72-74. Preferably, the CpG is a CpG-A ODN.

- 25 Preferably, the CpG oligonucleotide is constructed so that the 5' end is accessible for receptor recognition. Optionally, two CpG oligonucleotide sequences may be attached at their 3' ends to form "immunomers". See, for example, refs. 71 & 75-77.

- Bacterial ADP-ribosylating toxins and detoxified derivatives thereof may be used as adjuvants in the invention. Preferably, the protein is derived from *E.coli* (*E.coli* heat labile enterotoxin "LT"), cholera
30 ("CT"), or pertussis ("PT"). The use of detoxified ADP-ribosylating toxins as mucosal adjuvants is described in ref. 78 and as parenteral adjuvants in ref. 79. The toxin or toxoid is preferably in the form of a holotoxin, comprising both A and B subunits. Preferably, the A subunit contains a detoxifying mutation; preferably the B subunit is not mutated. Preferably, the adjuvant is a detoxified LT mutant such as LT-K63, LT-R72, and LT-G192. The use of ADP-ribosylating toxins and
35 detoxified derivatives thereof, particularly LT-K63 and LT-R72, as adjuvants can be found in refs. 80-

87. Numerical reference for amino acid substitutions is preferably based on the alignments of the A and B subunits of ADP-ribosylating toxins set forth in ref. 88, specifically incorporated herein by reference in its entirety.

F. Human immunomodulators

- 5 Human immunomodulators suitable for use as adjuvants in the invention include cytokines, such as interleukins (*e.g.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12 [89], *etc.*) [90], interferons (*e.g.* interferon- γ), macrophage colony stimulating factor, and tumor necrosis factor.

G. Bioadhesives and Mucoadhesives

- 10 Bioadhesives and mucoadhesives may also be used as adjuvants in the invention. Suitable bioadhesives include esterified hyaluronic acid microspheres [91] or mucoadhesives such as cross-linked derivatives of poly(acrylic acid), polyvinyl alcohol, polyvinyl pyrrolidone, polysaccharides and carboxymethylcellulose. Chitosan and derivatives thereof may also be used as adjuvants in the invention [92].

H. Microparticles

- 15 Microparticles may also be used as adjuvants in the invention. Microparticles (*i.e.* a particle of ~100nm to ~150 μ m in diameter, more preferably ~200nm to ~30 μ m in diameter, and most preferably ~500nm to ~10 μ m in diameter) formed from materials that are biodegradable and non-toxic (*e.g.* a poly(α -hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone, *etc.*), with poly(lactide-co-glycolide) are preferred, optionally treated to have a
20 negatively-charged surface (*e.g.* with SDS) or a positively-charged surface (*e.g.* with a cationic detergent, such as CTAB).

I. Liposomes (Chapters 13 & 14 of ref. 40)

Examples of liposome formulations suitable for use as adjuvants are described in refs. 93-95.

J. Polyoxyethylene ether and polyoxyethylene ester formulations

- 25 Adjuvants suitable for use in the invention include polyoxyethylene ethers and polyoxyethylene esters [96]. Such formulations further include polyoxyethylene sorbitan ester surfactants in combination with an octoxynol [97] as well as polyoxyethylene alkyl ethers or ester surfactants in combination with at least one additional non-ionic surfactant such as an octoxynol [98]. Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether
30 (laureth 9), polyoxyethylene-9-stearyl ether, polyoxyethylene-8-stearyl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether.

K. Polyphosphazene (PCPP)

PCPP formulations are described, for example, in refs. 99 and 100.

L. Muramyl peptides

Examples of muramyl peptides suitable for use as adjuvants in the invention include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), and N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE).

M. Imidazoquinolone Compounds.

Examples of imidazoquinolone compounds suitable for use adjuvants in the invention include Imiquamod and its homologues (e.g. "Resiquimod 3M"), described further in refs. 101 and 102.

The invention may also comprise combinations of aspects of one or more of the adjuvants identified above. For example, the following adjuvant compositions may be used in the invention: (1) a saponin and an oil-in-water emulsion [103]; (2) a saponin (e.g. QS21) + a non-toxic LPS derivative (e.g. 3dMPL) [104]; (3) a saponin (e.g. QS21) + a non-toxic LPS derivative (e.g. 3dMPL) + a cholesterol; (4) a saponin (e.g. QS21) + 3dMPL + IL-12 (optionally + a sterol) [105]; (5) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions [106]; (6) SAF, containing 10% squalene, 0.4% Tween 80™, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion. (7) Ribi™ adjuvant system (RAS), (Ribi Immunochem) containing 2% squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); and (8) one or more mineral salts (such as an aluminum salt) + a non-toxic derivative of LPS (such as 3dMPL).

Other substances that act as immunostimulating agents are disclosed in chapter 7 of ref. 40.

The use of an aluminium hydroxide or aluminium phosphate adjuvant is particularly preferred, and antigens are generally adsorbed to these salts. Aluminium hydroxide is preferably avoided as an adjuvant if the composition includes a Hib antigen. Where an aluminium phosphate it used and desired not to adsorb an antigen to the adjuvant, this is favoured by including free phosphate ions in solution (e.g. by the use of a phosphate buffer). Prevention of adsorption can also be achieved by selecting the correct pH during antigen/adjuvant mixing, an adjuvant with an appropriate point of zero charge, and an appropriate order of mixing for different antigens in a composition [107].

Calcium phosphate is another preferred adjuvant.

Further antigens

Compositions of the invention contain five basic meningococcal protein antigens. They may also include further antigens, although it may contain no meningococcal protein antigens other than the five basic antigens. Further antigens for inclusion may be, for example:

- a saccharide antigen from *Haemophilus influenzae* B.

- a saccharide antigen from *N.meningitidis* serogroup A, C, W135 and/or Y, such as the oligosaccharide disclosed in ref. 108 from serogroup C or the oligosaccharides of ref. 109.
- a saccharide antigen from *Streptococcus pneumoniae* [e.g. refs. 110 to 112].
- an antigen from hepatitis A virus, such as inactivated virus [e.g. 113, 114].
- 5 — an antigen from hepatitis B virus, such as the surface and/or core antigens [e.g. 114, 115].
- a diphtheria antigen, such as a diphtheria toxoid [e.g. chapter 3 of ref. 116] e.g. the CRM₁₉₇ mutant [e.g. 117].
- a tetanus antigen, such as a tetanus toxoid [e.g. chapter 4 of ref. 116].
- an antigen from *Bordetella pertussis*, such as pertussis holotoxin (PT) and filamentous
- 10 haemagglutinin (FHA) from *B.pertussis*, optionally also in combination with pertactin and/or agglutinogens 2 and 3 [e.g. refs. 118 & 119]. Cellular pertussis antigen may be used.
- an outer-membrane vesicle (OMV) preparation from *N.meningitidis* serogroup B, such as those disclosed in refs. 120, 121, 122 etc.
- polio antigen(s) [e.g. 123, 124] such as OPV or, preferably, IPV.
- 15 The composition may comprise one or more of these further antigens. Antigens will typically be present at a concentration of at least 1µg/ml each. In general, the concentration of any given antigen will be sufficient to elicit an immune response against that antigen. It is preferred that the protective efficacy of individual saccharide antigens is not removed by combining them, although actual immunogenicity (e.g. ELISA titres) may be reduced.
- 20 Where a diphtheria antigen is included in the composition it is preferred also to include tetanus antigen and pertussis antigens. Similarly, where a tetanus antigen is included it is preferred also to include diphtheria and pertussis antigens. Similarly, where a pertussis antigen is included it is preferred also to include diphtheria and tetanus antigens. Such DTP combinations can be used to reconstitute lyophilised conjugates.
- 25 Where a saccharide or carbohydrate antigen is used, it is preferably conjugated to a carrier protein in order to enhance immunogenicity. Covalent conjugation is a well known technique. Preferred carrier proteins are bacterial toxins or toxoids, such as diphtheria toxoid or tetanus toxoid. The CRM₁₉₇ diphtheria toxoid [125-127] is particularly preferred. Other suitable carrier proteins include the
- 30 *N.meningitidis* outer membrane protein [128], synthetic peptides [129,130], heat shock proteins [131,132], pertussis proteins [133,134], cytokines [135], lymphokines [135], hormones [135], growth factors [135], artificial proteins comprising multiple human CD4⁺ T cell epitopes from various pathogen-derived antigens [136], protein D from *H.influenzae* [137,138], pneumococcal surface protein PspA [139], iron-uptake proteins [140], toxin A or B from *C.difficile* [141], etc. Preferred carriers are diphtheria toxoid, tetanus toxoid, *H.influenzae* protein D, and CRM₁₉₇.
- 35 Any suitable conjugation reaction can be used, with any suitable linker where necessary. The saccharide will typically be activated or functionalised prior to conjugation. Activation may involve,

for example, cyanylating reagents such as CDAP (*e.g.* 1-cyano-4-dimethylamino pyridinium tetrafluoroborate [142,143,*etc.*]). Other suitable techniques use carbodiimides, hydrazides, active esters, norborane, p-nitrobenzoic acid, N-hydroxysuccinimide, S-NHS, EDC, TSTU. Linkages via a linker group may be made using any known procedure, for example, the procedures described in references 144 and 145. One type of linkage involves reductive amination of the polysaccharide, coupling the resulting amino group with one end of an adipic acid linker group, and then coupling a protein to the other end of the adipic acid linker group [146,147]. Other linkers include B-propionamido [148], nitrophenyl-ethylamine [149], haloacyl halides [150], glycosidic linkages [151], 6-aminocaproic acid [152], ADH [153], C₄ to C₁₂ moieties [154] *etc.* As an alternative to using a linker, direct linkage can be used. Direct linkages to the protein may comprise oxidation of the polysaccharide followed by reductive amination with the protein, as described in, for example, references 155 and 156.

Toxic protein antigens may be detoxified where necessary (*e.g.* detoxification of pertussis toxin by chemical and/or genetic means [119]).

As an alternative to using protein antigens in the composition of the invention, nucleic acid encoding the antigen may be used [*e.g.* refs. 157 to 165]. Protein components of the compositions of the invention may thus be replaced by nucleic acid (preferably DNA *e.g.* in the form of a plasmid) that encodes the protein. Similarly, compositions of the invention may comprise proteins which mimic saccharide antigens *e.g.* mimotopes [166] or anti-idiotypic antibodies. These may replace individual saccharide components, or may supplement them. As an example, the vaccine may comprise a peptide mimic of the MenC [167] or the MenA [168] capsular polysaccharide in place of the saccharide itself.

Particularly preferred compositions of the invention include one, two or three of: (a) saccharide antigens from meningococcus serogroups Y, W135, C and (optionally) A; (b) a saccharide antigen from *Haemophilus influenzae* type B; and/or (c) an antigen from *Streptococcus pneumoniae*. A composition comprising the serogroup B antigens and a Hib conjugate is particularly preferred.

General

The term “comprising” encompasses “including” as well as “consisting” *e.g.* a composition “comprising” X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term “about” in relation to a numerical value *x* means, for example, $x \pm 10\%$.

The word “substantially” does not exclude “completely” *e.g.* a composition which is “substantially free” from Y may be completely free from Y. Where necessary, the word “substantially” may be omitted from the definition of the invention.

References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of reference 169. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is taught in reference 170.

Sequences included to facilitate cloning or purification, *etc.*, do not necessarily contribute to the invention and may be omitted or removed.

- 10 This invention does not include within its scope any of the 287 or App fragments disclosed in the prior art *e.g.* in the documents cited herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1 and 2 are western blots using anti-287 showing the effect of lactoferrin on (1) purified protein 287 or (2) meningococcal bacteria.

- 15 Figures 3 and 4 show results of serum bactericidal assays using anti-287 serum. Bacteria were either treated (■) or untreated (♦) with lactoferrin. The 287 used to generate the serum was adjuvated either with aluminum hydroxide (Figure 3) or with complete Freund's adjuvant (Figure 4).

Figure 5 shows results of serum bactericidal assays using anti-NadA serum (▲ & ●) or SEAM3 (■ & ♦). Bacteria were either treated (■ & ●) or untreated (♦ & ▲) with lactoferrin.

- 20 Figures 6 and 7 are western blots using anti-App showing the effect of lactoferrin on (6) purified App or (7) meningococcal bacteria. Asterisks show degradation products.

Figure 8 is a western blot using anti-GNA1870 showing the effect of lactoferrin on meningococci.

MODES FOR CARRYING OUT THE INVENTION

Protein 287

- 25 An example of a full length 287 sequence is given in SEQ ID NO: 2. 0.5µg of 287 was mixed with 10µg human lactoferrin in a 20µl reaction volume. The mixture was incubated at 37°C for 2 hours, and 10µl of the mixture was then analysed by SDS-PAGE followed by a western blot using rabbit anti-287 (1:8000 dilution). The results are shown in Figure 1. The lanes show: (1) 287 in Tris buffer at room temperature; (2) 287 in Tris buffer at 37°C; (3) 287 in Tris buffer with lactoferrin at 37°C; and (4) lactoferrin in PBS buffer at 37°C. Lane 3 shows definite cleavage of protein 287. The sizes of the fragments (~40kDa and ~19kDa) are consistent with cleavage at the Arg-rich region in 287.
- 30

As a further control, the inhibitor decanoyl-RVKR-chloromethylketone was included in the reaction mixture. Cleavage was inhibited in the presence of this compound.

The effect of lactoferrin on meningococcal cells was also investigated. 1ml of log phase culture (OD=0.5) of strain 2996 was washed with Tris buffered saline and then incubated with 120µl of 1mg/ml lactoferrin for 2 hours at 37°C. The mixture was centrifuged and the cell pellet and supernatant were analysed by SDS-PAGE as described above. Results are shown in Figure 2.

- 5 Cleavage is seen, with the ~19kDa fragment being seen only in the supernatant. Thus the cleavage seems to release the C-terminal region of the protein into the supernatant.

The ~19kDa fragment of cleavage was subjected to N-terminus sequences. Two different N-termini 9mer sequences were seen: SLPAEMPLI (~70%) and RSLPAEMPL (~30%). These sequences are immediately downstream of the Arg-rich region in SEQ ID NO: 2 and differ only by one amino acid:

10 MFERSVIAMACIFALSACGGGGGGSPDVKSADTLSPKPAAPVVAEKETEVEDAPQAGSQGGAPSTQGSQDMAAVSAENTGNGGAATTDKP
KNEDEGPQNDMPQNSAESANQTGNNQPADSSDSAPASNPAFANGGSNFRVLDLANGVLIDGPSQNTLTTHCKGDSNGDNLDEEAPSKSE
FENLNESERIEKYKKGKSDKFTNLVATAVQANGTNKYVVIYKDKSASSSSARFRRSARSRSSLPAEMPLIIPVNQADTLIVDGEAVSLTGH
SGNIFAPEGNRYRLTYGAEKLPGGSYALRVQGEPAKGEMLAGTAVYNGEVLHFEHTENGRPYPTRGRFAAKVDFGSKSVGLIDSGDDLHMG
15 TQKFKAIDGNGFKGTWTENGDDVSGRFYGPAGEEVAGKYSYRPTDAEKGGFGVFAGKKEQD

- 287 protein is an effective antigen for raising bactericidal antibody responses. The effect of lactoferrin treatment on this activity was tested. After treating meningococcus strain 2996 with human lactoferrin or with PBS buffer for 2 hours at 37°C, the bactericidal assay was performed using serum obtained using uncleaved ΔG287 (adjuvanted either with alum or CFA). As a negative control, antiserum to NadA was used. As a positive control, the SEAM3 antibody was used.

Figures 3 and 4 show that anti-287 serum is less effective against lactoferrin-treated bacteria than against the untreated bacteria. In both cases the serum was active for three further serial dilutions against the untreated bacteria. In contrast, lactoferrin treatment had no effect on the bactericidal activity of anti-NadA serum or of the SEAM3 antibody (Figure 5).

25 App

- An example of a full length App sequence is given in SEQ ID NO: 1. 0.5µg of recombinant App was incubated at 37°C for 2 hours with 10µg human lactoferrin in a 20µl reaction volume. Western blot analysis using anti-App (1:1000 dilution) showed that the App antigen was cleaved in different fragments (Figure 6). In Figure 6, the lanes are: (1) App + PBS buffer at 25°C; (2) App + PBS buffer at 37°C; (3) App + Lf at 37°C; (4) Lf + PBS.

In additional experiments, *N.meningitidis* strain MC58 bacteria were resuspended in human lactoferrin and incubated for 2 hours at 37°C. After incubation, whole cells lysates were examined by western immunoblotting using an anti-App antiserum. As shown in Figure 7, treatment with lactoferrin degraded the protein generating a fragment of ~97 kDa in the bacterial cell pellet.

- 35 To address the specificity of the interaction between Lf and meningococcal surface proteins, the effect of Lf treatment on GNA1870 [17] surface-exposed lipoprotein was tested. As shown in Figure 8, lactoferrin treatment under conditions which degrade App had no effect on GNA1870

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

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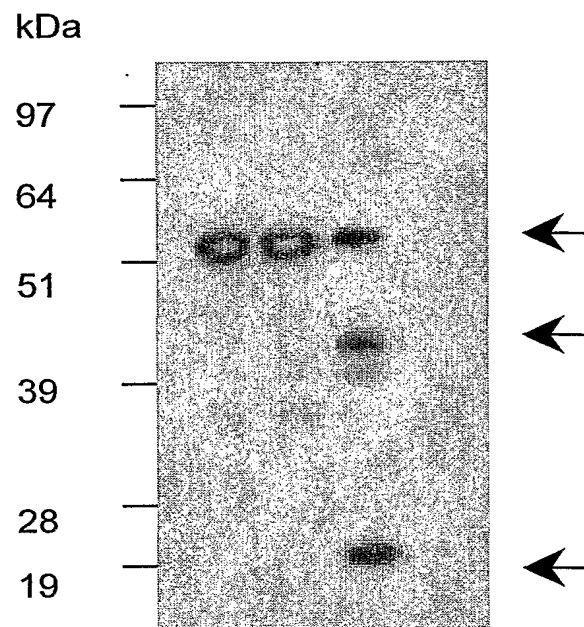
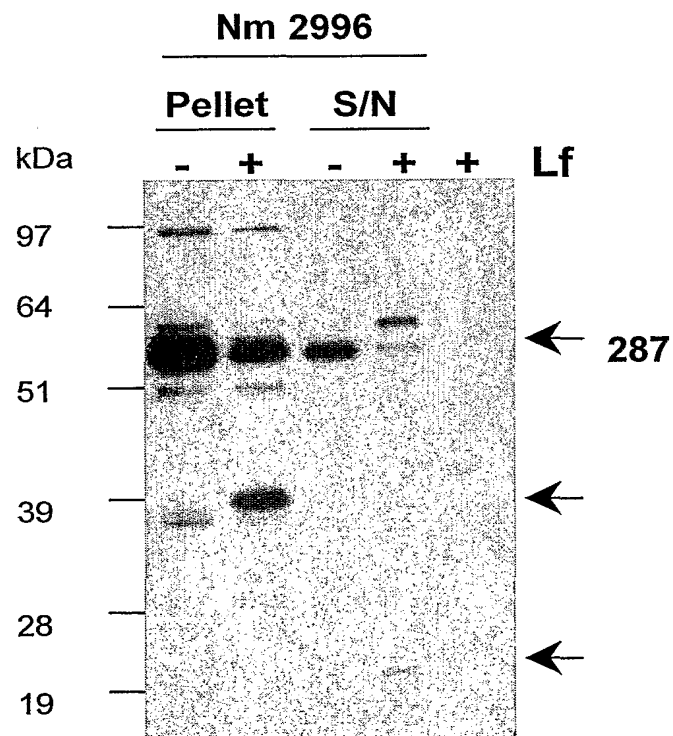
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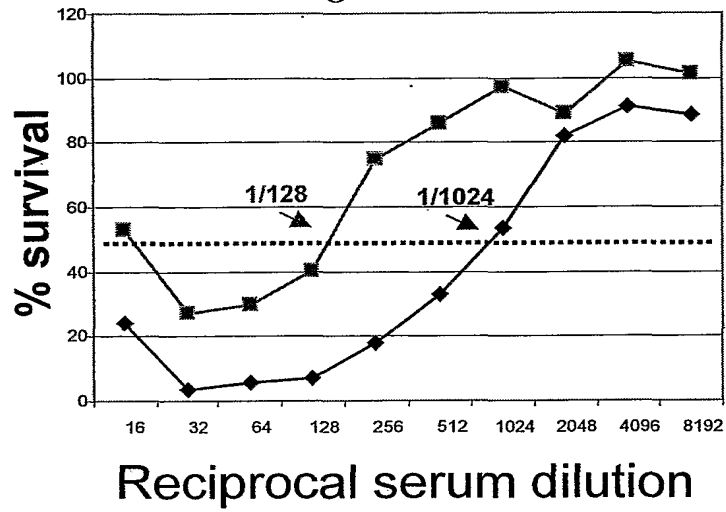
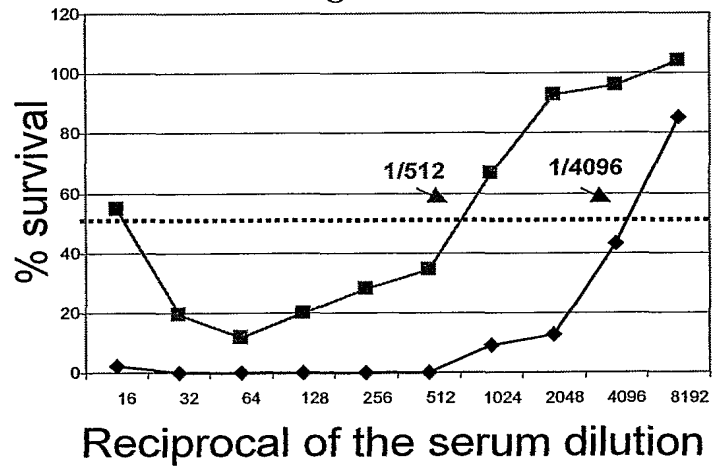
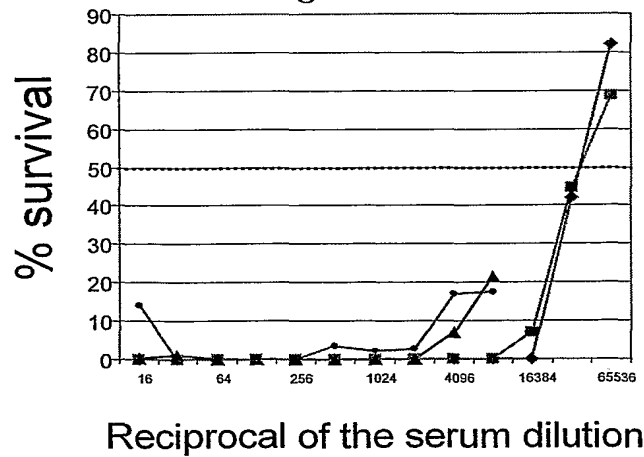
CLAIMS

1. A method for cleaving a neisserial polypeptide, comprising the step of mixing the polypeptide with a lactoferrin enzyme.
2. The method of claim 1, wherein the neisserial polypeptide is a meningococcal polypeptide.
- 5 3. The method of claim 2, wherein the meningococcal polypeptide is a 'NadA', '936', '953', '287', 'App' or 'NarE' polypeptide.
4. The method of claim 3, wherein polypeptide is a '287' polypeptide, which polypeptide has 50% or more identity to SEQ ID NO: 2, and/or comprises at least 7 consecutive amino acids from SEQ ID NO: 2.
- 10 5. The method of claim 3, wherein polypeptide is a 'App' polypeptide, , which polypeptide has 50% or more identity to SEQ ID NO: 1, and/or comprises at least 7 consecutive amino acids from SEQ ID NO: 1.
6. The method of any preceding claim, wherein the lactoferrin is human lactoferrin.
7. A polypeptide, which polypeptide is a cleavage product obtainable by the process of claim 1.
- 15 8. The polypeptide of claim 7, wherein the polypeptide comprises an amino acid sequence of formula -A1-B1-C1-, where: A1 is an optional arginine residue; B1 is a sequence with 50% or more sequence identity to SEQ ID NO: 7; and C1 is any amino acid sequence.
9. The polypeptide of claim 8, wherein sequence C1 has $\geq 50\%$ sequence identity to SEQ ID NO: 8.
10. The polypeptide of claim 8 or claim 9, wherein the -A1-B1-C1- sequence is at the N-terminus of
20 the polypeptide, at the C-terminus of the polypeptide, or at neither.
11. The polypeptide of any one of claims 8 to 10, wherein the -C1- moiety consists of at least 10 amino acids.
12. The polypeptide of any one of claims 8 to 11, wherein -B1- is SEQ ID NO: 7 and/or -C1- is SEQ ID NO: 8.
- 25 13. The polypeptide of claim 7, wherein the polypeptide comprises an amino acid sequence of formula -A2-B2-, where: B2 is an optional arginine residue; and A2 is a sequence with 50% or more sequence identity to SEQ ID NO: 9 and/or SEQ ID NO: 10.
14. The polypeptide of claim 13, wherein the -A2-B2- sequence is at the N-terminus of the polypeptide, at the C-terminus of the polypeptide, or at neither.
- 30 15. The polypeptide of any one of claims 13 to 14, wherein the -A2- moiety consists of at least 10 amino acids.
16. The polypeptide of any one of claims 13 to 15, wherein the -A2- moiety is SEQ ID NO: 9.
17. Nucleic acid encoding the polypeptide of any one of claims 7 to 16.

1/3

Figure 1**Figure 2**

2/3

Figure 3*Figure 4**Figure 5*

3/3

Figure 6

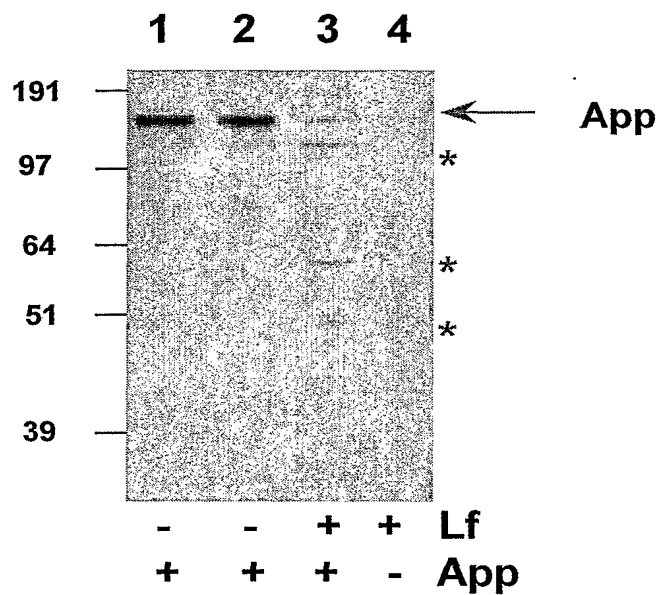


Figure 7

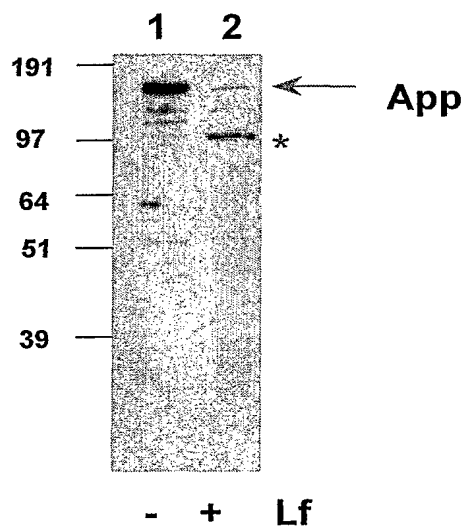
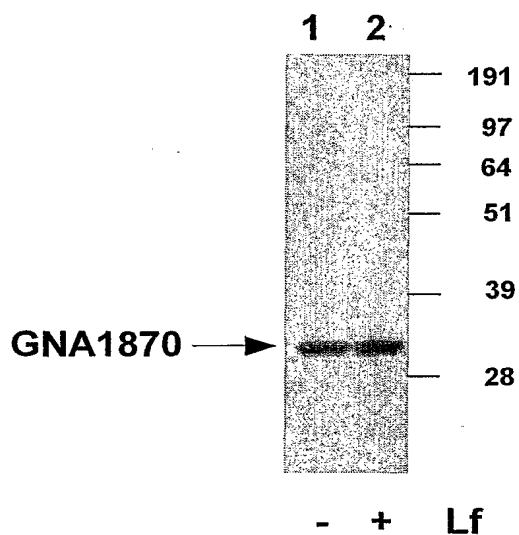


Figure 8



SEQUENCE LISTING

SEQ ID NO: 1 – App

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 5 RLHKFVTDAPVEMTSYMDGRKYIDQNNYPDRVRIGAGRQYWRSEDEPNNRESSYHIASAYSWLVGNTFAQNGSGGGTV
 NLGSEKIKHSPYGFLLPTGGSGFDGSGSPMFIYDAQKQKWLINGVLQGTGNPYIGKSNGFQLVRKDWFYDEIFAGDTHSVFYEP
 RQNGKYSFNDDNNGTGKINAKHEHNSLPNRLKTRTVQLEFNVSLSETAREPVYHAAGGVNSYRPRLNNGENISFIDEGKGEL
 ILTSNINQGAGGLYFQGDFTVSPENNETWQAGVHISEDSTVTWKVNGVANDRLSKIGKGLHVQAKGENQGSISVGDGTV
 ILDQQADDKGGKQAFSEIGLVSGRGTVQLNADNQFNPKLYFGFRGGRDLNGHSLSFHRIQNTDEGAMIVNHNQDKESTV
 10 TITGNKDIAATTGNNNSLDSKKEIAYNGWFGKDTTKTNGRLNLVYQPAAEDRTLLLSGGTNLNGNITQTNGKLFFSGRPTP
 HAYNHLNDHWSQKEGIPRGEIVWDNDWINRTFKAENFQIKGGQAVVSRNVAKVKGDWHLSNHAQAVFGVAPHQSHTICTRS
 DWTGLTNCVEKITITDDKVIASLTKTDISGNVDLADHAHLNLTGLATLNGNLSANGDTRYTVSHNATQNGNLSLVGNAQATF
 NQATLNGNTSASGNASFNLSDAHVQNGSLTSGNAKANVSHSALNGNVSLADKAVFHFESSRFTGQISGGKDTALHLKDSE
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 15 FMSELFGYRSDKLKLAESSEGTYT LAVNNTGNEPASLEQLTVVEGKDNKPLSENLFNLQNEHVDAGAWRYQLIRKDGFR
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SEQ ID NO: 2 – 287

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 KGDSCNGDNLDEEAPSKSEFENLNESEKIEYKKDGKSDKFTNLVATAVQANGTNKYVIIYKDKSASSSSARFRRSARS
 25 RSLPAEMPLIPVNQADTLIVDGEAVSLTGHSNIFAPEGNYRYLTYGAEKLPGGSYALRVQGEPAKGEMLAGTAVYNGEVL
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SEQ ID NO: 3 – Nada

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 30 DFKGLGLKKVVTNLTKTVNENKQNVDAKVKAASEIEKLTTKLADTDAALADTDAALDETTNALNKLGENITTFEETKTN
 IVKIDEKLEAVADTVDKHAEAFNDIADSLDETNTKADEAVKTANEAKQTAEETKQNVDAKVKAETAAGKAEAAAGTANTA
 ADKAEAVAAKVTDIKADIATNKADIAKNSARIDSLDKNVANLRKETRQGLAEQAALSGLFQPYNVGRFNVTAAVGGYKSES
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SEQ ID NO: 4 – 936

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SEQ ID NO: 5 – 953

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 GMDYLVNVMGTSVRIDIQIEAAKQ

SEQ ID NO: 6 – NarE

MGNFLYRGISCQQDEQNNQGLKPKGNKAEVAIRYDGKFKYDGKATHGPSVKNAVYAHQIETGLYDGCYISTTTDKIEIAKKE
 45 ATSSGIENGYIYVLNRDLFGQYSIFEYEVEHPENPNEKEVTIRAEDCGCIPEEVIIAKELIEIN

SEQ ID NO: 7 – 10mer fragment of SEQ ID NO: 2

SLPAEMPLIP

SEQ ID NO: 8 – Fragment of SEQ ID NO: 2

VNQADTLIVDGEAVSLTGHSGNIFAPEGNYRYLTYGAEKLPGGSYALRVQGEPAKGEMLAGTAVYNGEVLHFHTENGRYPY
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FGVFAGKKEQD

5 **SEQ ID NO: 9 – Fragment of SEQ ID NO: 2**

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SEQ ID NO: 10 – Fragment of SEQ ID NO: 2

- 10 SPDVKSADTLSPKPAAPVVAEKETEVEKEDAPQAGSQGQAPSTQGSQDMAAVSAENTGNGGAATTDKPKNEDEGPQNDMPQ
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NESERIEKYKKDGKSDKFTNLVATAVQANGTNKYVILYKDKSASSSSARFRRSARSR

SEQ ID NO: 11 – TonBR

- 15 MTRFKYSLFAALLPVYAQADVSVSDPKPQESTELPTITVTADRTASSNDGYTVSGTHTPLGLPMTLREIPQSVSVITSQ
QMRDQNIKTLDRLALQATGTSRQIYGSDRAGYNLFARGSRANYQINGIPVADALADTGNANTAAAYERVEVVRGVAGLLD
GTGEPSATVNLVRKRLTRKPLFEVRAEAGNRKHFGLDADVSGSLNTEGTLRGRLVSTFGRGDSWRRRERSRDAELYGILEY
DIAPQTRVHAGMDYQQAQKETADAPLSYAVYDSQGYATAFGPKDNPATNWANSRHRALNLFAGIEHRFNQDWKLKAEYDYTR
SRFRQPYGVAGVLSIDHNTAATDLIPGYWHADPRTHSASVSLIGKYRLFGREHDLIAGINGYKYASNKYGERSIIPNAIPN
AYEFSRTGAYPQPASFAQTIPQYGTTRRQIGGYLATRFRAADNLSLILGGRYTRYRTGSYDSRTQGMYVSANRFTPYTGIV
20 FDLTGNLSLYGSYSSLFVPPSQKDEHGSYLKPVGTGNLEAGIKGEWLEGRNLASAAYVRARKNNLATAAGRDPSGNTYYRA
ANQAKTHGWEIEVGGRIPEWQIQAGYSQSKTRDQDGSRLNPDSPERSFKLFTAYHFAPEAPSGWTIGAGVRWQSETHTD
PATLRIPNPAKARAADNSRQKAYAVADIMARYRENPRALSLNVDNLFNKHYRTQPDHRHSYGALRTVNAAFTYRFK

SEQ ID NO: 12 – Fragment of SEQ ID NO: 1

SLLSVTPPTS

25 **SEQ ID NO: 13 – Fragment of SEQ ID NO: 1**

- VESRFNTLTVNGKLNQGTFRFMSELFYRSDKLKLAESSEGYTLAVNNTGNEPASLEQLTVVEGKDNKPLSENLFNLQ
NEHVDAGAWRYQLIRKDGELFRLHNPVKEQELSDKLKGAELKQAEKDNAQSLDALIAAGRDAVEKTESVAEPARQAGGENV
GIMQAEKKRVQADKDTALAKQREAEATPATTAFFRARRARRDLPLQLPQPQPQQRDLISRYANGLSEFSATLNSVFA
VQDELDRVFAEDRRNAVWTSIGIRDTKHYSQDFRAYRQOTDLRQIGMQKNLGSGRVGILFHSNRTENTFDDGIGNSARLAH
30 GAVFGQYGIDRFYIGISAGAGFSSGSLSDGIGGKIRRRVLHYGIQARYRAGFGGFGIEPHIGATRYFVQKADYRYENVNIA
TPGLAFNRYRAGIKADYSFKPAQHISITPYLSLSYTDAAASGKVRTRVNTAVLAQDFGKTRSAEWGVNAEIKGFTLSLHAAA
AKGPQLEAQHSAGIKLGYRW

SEQ ID NO: 14 – Fragment of SEQ ID NO: 1

DLPQLQPQPQPQPQ

35 **SEQ ID NO: 15 – Fragment of SEQ ID NO: 1**

RDLSRYANGLSEFSATLNSVFAVQDELDRVFAEDRRNAVWTSIGIRDTKHYSQDFRAYRQOTDLRQIGMQKNLGSGRVG
ILFHSNRTENTFDDGIGNSARLAHGAVFGQYGIDRFYIGISAGAGFSSGSLSDGIGGKIRRRVLHYGIQARYRAGFGGFGI
EPHIGATRYFVQKADYRYENVNIATPGLAFNRYRAGIKADYSFKPAQHISITPYLSLSYTDAAASGKVRTRVNTAVLAQDFG
KTRSAEWGVNAEIKGFTLSLHAAAAKGPQLEAQHSAGIKLGYRW

40 **SEQ ID NO: 16 – Fragment of SEQ ID NO: 1**

- MKTTDKRTTETHRKAPKTGRIRFSPAYLAICLSFGILPQAWAGHTYFGINYQYYRDFAEKNGKFAVGAKDIEVYNKKGELV
GKSMTKAPMIDFSVSRNGVAALVGDQYIVSAHNGGYNVDFGAEGRNPDQHRFTYKIVKRNNYKAGTKGHPYGGDYHMP
RLHKFVTDAPVEMTSYMDGRKYIDQNNYPDRVRIGAGRQYWRSDDEPNNRESSYHIASAYSWLVGNGTFAQNGSGGGTV
NLGSEKIKHSPYGFLLPTGGSFGDSGSPMFIYDAQKQKWLINGVLQTNPYIGKSNFQLVRKDWFYDEIFAGDTHSVFYEP
45 RQNGKYSFNDDNNGTGKINAKHEHNSLPNRLKTRTVQLFNVSLSETAREPVYHAAGGVNSYRPRLNNGENISFIDEKGKEL

ILTSNINQGAGGLYFQGDFTVSPENNETWQAGVHISEDSTVTWKVNGVANDRLSKI GKGLHVQAKGENQGSISVGDGTV
ILDQQADDKGGKQAFSEIGLVSGRGTVQLNADNQFNPKLYFGFRGGRLDLNGHSLSFHRIQNTDEGAMIVNHNQDKESTV
TITGNKDIAATTGNNNSLDSKKEIAYNGWFGEKDTTKTNGRLNLVYQPAEDRTL LLSGGTNLNGNITQTNGKLFFSGRPTP
HAYNHLNDHWSQKEGIPRGEIVWDNDWINRTFKAENFQIKGGQAVVSRNVAKVKGDWHL SNHAQAVFGVAPHQSHTICTRS
5 DWTGLTNCVEKTITDDKVIASLTKTDISGNVDLADHAHLNLTGLATLNGNLSANGDTRYTVSHNATQNGNLSLVGNAQATF
NQATLNGNTSASGNASFNLSDHAVQNGSLT LSGNAKANVSHSALNGNVSLADKAVFHFESSRFTGQISGGKDTALHLKDSE
WTLPSGTELGNLNLNDNATITLNSAYRHDAAGAQTGSATDAPRRRSRRSR

SEQ ID NO: 17 – Fragment of SEQ ID NO: 1

MKTTDKRTTETHRKAPKTGRIRFSPAYLAICLSFGILPQAWAGHTYFGINYQYYRDFAEKNGKFAVGAKDIEVYNKKGELV
10 GKSMTKAPMIDFSVSRNGVAALVG DQYIVSVAHNGGYNNVDFGAEGRPDQHRFTYKIVKRNNYKAGTKGHPYGGDYHMP
RLHKFVTD AEPVEMTSYMDGRKYIDQNNYPDRVRIGAGRQYWRSEDEPNNRESSYHIASAYSWLVG GNTFAQNGSGGGTV
NLGSEKIKHSPYGF LPTGGSGFGDSGSPMFIYDAQKQKWLINGVLQTGNPYIGKSNGFQLVRKDWFYDEIFAGDTHSVFYEP
RQNGKYSFNDDNNGTGKINAKHEHNSLPNRLKTRTVQLFNVSLSETAREPVYHAAGGVNSYRPRLNNGENISFIDEKGEL
15 ILTSNINQGAGGLYFQGDFTVSPENNETWQAGVHISEDSTVTWKVNGVANDRLSKI GKGLHVQAKGENQGSISVGDGTV
ILDQQADDKGGKQAFSEIGLVSGRGTVQLNADNQFNPKLYFGFRGGRLDLNGHSLSFHRIQNTDEGAMIVNHNQDKESTV
TITGNKDIAATTGNNNSLDSKKEIAYNGWFGEKDTTKTNGRLNLVYQPAEDRTL LLSGGTNLNGNITQTNGKLFFSGRPTP
HAYNHLNDHWSQKEGIPRGEIVWDNDWINRTFKAENFQIKGGQAVVSRNVAKVKGDWHL SNHAQAVFGVAPHQSHTICTRS
DWTGLTNCVEKTITDDKVIASLTKTDISGNVDLADHAHLNLTGLATLNGNLSANGDTRYTVSHNATQNGNLSLVGNAQATF
20 NQATLNGNTSASGNASFNLSDHAVQNGSLT LSGNAKANVSHSALNGNVSLADKAVFHFESSRFTGQISGGKDTALHLKDSE
WTLPSGTELGNLNLNDNATITLNSAYRHDAAGAQTGSATDAPRRRSRRSRRLSVTPPTSVE SRFNTLT VNGKLNQGTFR
FMSELFGYRSDKLKLAESSEGYTLAVNNTGNEPASLEQLTVVEGKDNKPLSENLF T LQNEHVDAGAWRYQLIRKDG EFR
LHNPVKEQELSDKLGAEAKKQAEKDNAQSLDALIAAGRDAVEKTESVAEPARQAGGENVGIMQAE EKKRVQADKDTALA
KQREAE TRPATTAFPRARRAR

SEQ ID NO: 18 – Fragment of SEQ ID NO: 2

25 KSASSSSARF

SEQ ID NO: 19 – Fragment of SEQ ID NO: 1

AQTGSATDAP

SEQ ID NO: 20 – Fragment of SEQ ID NO: 1

ETRPATTAFP

30 **SEQ ID NO: 21 – Fragment of SEQ ID NO: 2**

RRSARSRR

SEQ ID NO: 22 – Fragment of SEQ ID NO: 1

RRRSRRSRR

SEQ ID NO: 23 – Fragment of SEQ ID NO: 1

35 RARRARR

SEQ ID NO: 24 – Fragment of SEQ ID NO: 2

SLPAEMPLI

SEQ ID NO: 25 – Fragment of SEQ ID NO: 2

RSLPAEMPL